

Claims

SUB A'
cont'd

1. A method for identifying novel subunits of the human Anaphase Promoting Complex (APC), characterized by the steps
 - a) replacing in a cell of the budding yeast *Saccharomyces cerevisiae* one or more endogenous genes encoding a known APC subunit by epitope-tagged versions of said genes or transforming the cell with a vector containing the corresponding epitope-tagged cDNA(s),
 - b) growing yeast cells obtained in a) and preparing a protein extract,
 - c) isolating the yeast APC by contacting the protein extract obtained in b) with an antibody directed against the epitope-tag,
 - d) isolating the antibody-bound yeast protein(s) and purifying them,
 - e) determining the sequence of the yeast protein(s),
 - f) identifying the human subunit(s) by comparing the sequence(s) of the yeast protein(s) obtained in e) and/or the DNA sequence encoding those proteins with published human sequences.
2. The method of claim 1, wherein the gene(s) used in step a) are selected from the group *APC1*, *CDC16*, *CDC23*, *CDC26*, *CDC27*, *APC2*, *APC5*, *APC4*, *APC9* and *APC11*.
3. A method for identifying novel subunits of the human Anaphase Promoting Complex (APC), characterized by the steps
 - a) replacing in a human cell one or more endogenous genes encoding known APC subunits by epitope-tagged versions of said genes or transforming the cell with a vector containing the corresponding epitope-tagged cDNA(s) and establishing a cell line,
 - b) growing the cell line obtained in a) and preparing a protein extract,
 - c) isolating the APC by contacting the protein extract obtained in b) with an antibody directed against the epitope-tag,
 - d) isolating the antibody-bound protein(s) and purifying them,
 - e) determining the sequence of the protein(s).

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4. The method of claim 3, wherein the gene(s) or the respective cDNAs used in step a) are selected from a group of genes that are homologs of the yeast genes *APC1*, *CDC16*, *CDC23*, *CDC26*, *CDC27*, *APC2*, *APC5*, *APC4*, *APC9* and *APC11*.
5. A method for producing recombinant APC, characterized in that cDNAs encoding APC subunits are expressed in a suitable host, said subunits are isolated, purified and allowed to assemble to form a functional APC.
6. The method of claim 5, wherein the cDNAs are expressed in a Baculovirus expression system.
7. The use of recombinant APC in a screening method for identifying substances that inhibit rapidly proliferating cells by interfering with the cells' entry into the subsequent cell cycle.
8. The use of claim 7 wherein said method comprises determining the effect of a substance on the APC's ability to ubiquitinate a substrate.
9. The use of claim 7 or 8, wherein the APC and the substrate are of human origin.
10. The use of claim 9, wherein the substrate is a recombinant cyclinB.

Sub A1
cont'd.
Add A2add
C1